

Analysis of Marijuana (delta 9 THC)

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1. Background

Marijuana History (derived), different appearances/textures,

2. Objective

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain marijuana (^ 9 THC).

3. Scope

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

4. Responsibility

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

{ DATE \@ "M/d/yyyy" }

Technical Reviewers will review each case and complete the comprehensive reviewer checklist. They will ensure that the chemist followed this SOP. The Technical Reviewer may perform the duties and responsibilities of the chemist.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

1. Related Documents

Cole, Michael, "The Analysis of Controlled Substances," London: John Wiley & Sons Ltd., 2003
Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.

Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3rd ed., 6 vols., New York: CRC Press, 2006.

Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2nd ed., London: The Pharmaceutical Press, 1986.

Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3rd ed., London: The Pharmaceutical Press, 2004.

Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.

Scientific Working Group for the Analysis of Seized Drug Recommendation, 6th ed., "Part III A & B, Methods of Analysis/Sampling of Seized Drug for Qualitative Analysis," July 2011

6. Definitions

GC w/ FID: Gas Chromatography with Flame Ionization Detector

GC/MS: Gas Chromatography/Mass Spectrometry

Gross Weight: The weight of both the substance and its container.

Net Weight: The weight of the substance only.

Cystolithic hairs: These are unicellular, sharply pointed curved, conical trichome with enlarged bases that contain deposits of calcium carbonate. The shapes of these hairs resemble "bear claws." They are found in greater abundance on the upper side of the leaf.

Glandular hairs: These are multicellular with conical trichomes that are long and slender. These hairs have a shiny appearance and a sticky touch due to the resin. The glandular hairs form on the surface of the leaf or flowering tops.

Gross morphological: These are types of characteristics of palmate arrangements, pinnate appearance, serrated edges of the leaflets, buds (with or without seeds) and if present stems and stalks.

7. Supplies, Equipment & Reagents

Supplies

{ DATE \@ "M/d/yyyy" }

GC columns

HP-1MS (Agilent, Cat # 19091S-933UI or equivalent)

HP-5MS (Agilent, Cat # 19091S-433UI or equivalent)

GC crimp vials

Clear (Agilent, 2mL, Cat # 5182-0543 or equivalent)

Amber (Agilent, 2mL, Cat # 5181-3376 or equivalent)

Clear (Agilent, 0.8mL, Cat# or equivalent)

Kimwipes

Pasteur pipette

Scissors

Spatula

Stirring rod

Teflon crimp (top) cap

Silver (Agilent, Cat # 5181-1210 or equivalent)

Blue (Agilent, Cat # 5181-1215 or equivalent)

Various Class A glassware

Beaker

Graduated cylinder

Volumetric flask (range 10mL to 500mL)

Volumetric pipette

Weighing dish (VWR, Anti-Static, Cat # 89106 or equivalent)

Weighing paper (VWR, Cat # 12578 or equivalent)

Porcelain dish

Adjusted pipette

Tweezer

Culture tubes

Equipment

Analytical Balance (range 0.0001g to 1.0g)

GC with FID (Agilent, Model # 7890 Series or equivalent)

GC/MS (Agilent, Model # 5975 Series or equivalent)

Stereomicroscope

Hot plate

Reagents

Chloroform (JT Baker, ACS Grade, Cat # 9180 or equivalent)

Vanillin, crystalline (Fisher, Cat # V-10 or equivalent)

Acetaldehyde, 99.5% (Acros Organics, Cat #

Hydrochloric acid

Petroleum ether

Deionized water (in-house)

Methanol (JT Baker, ACS Grade, Cat # 9070 or equivalent)

95% Ethanol

Standards

Methadone

⁹ THC

8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses. Lab coats are to be worn at all times in the unit, unless performing administrative duties.

9. Reagent/Standard Preparation

Modified Duquenois Levine Reagent

Dissolve 4.0g of vanillin in 2.5mL of acetaldehyde and bring to volume with 200mL of 95% ethanol. Mix the solution until completely dissolved.

THC Standard

Methadone Internal Standard Stock (ISTD) [0.50mg/mL]

A. Dissolve 25mg of methadone into a 500mL volumetric flask and bring to volume with methanol. Mix the solution until completely dissolved.

B. Pipette 50mL of methadone ISTD stock into a 100mL amber bottle using a volumetric pipette. [0.50mg/mL ISTD]

C. The remaining stock solution from A will be brought to volume with 500mL of methanol. [0.45mg/mL ISTD]

THC Stock Solution

Pour 2 vials of Δ^9 THC (10mg/mL) reference standard into a labeled conical tube. Mix the solution completely.

THC Quantitative Standards

[1.0mg/mL] Concentration of 1.0mg/mL of THC Standard

Pipette 1.0mL of Δ^9 THC stock solution into a 10mL volumetric flask and bring to volume with [0.50mg/mL] ISTD methadone stock solution (B).

[0.40mg/mL] Concentration of 0.40mg/mL of THC Standard

Pipette 4.0mL of [1.0mg/mL] THC standard into a 10mL volumetric flask and bring to volume with [0.45mg/mL] ISTD methadone stock solution (C).

[0.20mg/mL] Concentration of 0.20mg/mL of THC Standard

Pipette 1.0mL of [0.40mg/mL] THC standard into a 10mL volumetric flask and bring to volume with [0.45mg/mL] ISTD methadone stock solution (C).

10. Procedure

A. Evidence Handling

- i. Evidence Officer will randomly assign sample to a chemist.

- ii. The chemist will perform an evidentiary check on the sample. They will verify that the manila envelop, control card and the evidence correspond. They will observe the integrity evidence bag and its contents.
- iii. Once the sample has being verified, the chemist will take custody of the samples by signing out the evidence in the chain of custody logbook.
- iv. The sample will be brought to the chemist work area and stored in a secure manner at all times.
- v. Upon analysis of each sample, the chemist will document all observations on the Drug Analysis Form.
- vi. The information on the Drug Analysis Form will contain but not limited to the sample number, submitting agency, verification of the evidence gross weight, number of samples, container, description of sample, gross, package and net weight, ballistics notation, chemist notations and results, preliminary and confirmatory findings.

B. Sampling Plan (see chart)

- i. To be determine

C. Residues

- i. Attempt to scrape or remove sample from the device and place onto weighing paper or boat. Or rinse the device containing the sample with 1-2ml of the chloroform and place the extract into a beaker.
- ii. Transfer some of the sample or extract into a labeled residue vial for GC and GC/MS analysis. Residue samples should be dissolved or diluted in chloroform. Cap and seal the vial tightly.
- iii. Use the remaining sample or extract to perform the color test.

D. Macroscopic Identification

- i. Remove a portion of the sample and place into a clean porcelain dish.
- ii. Observe the sample by a visual examination.
- iii. Identify the gross morphological characteristics of the sample.
- iv. A positive macroscopic examination will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when sufficient characteristics are observed and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

E. Microscopic Identification

- i. Remove a portion of the sample and place into a clean porcelain dish.
- ii. Observe the sample at varying magnifications (approx. 10x – 40x) using a stereomicroscope.
- iii. Identify the presence of cystolithic hairs.
- iv. Identify the presence of glandular hairs.
- v. A positive macroscopic examination will be recorder on the Drug Analysis Form by the use of a plus (+). The result is considered positive when sufficient characteristics for both the cystolithic and glandular hairs are observed and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

F. Color Test (Modified Duquenois-Levine)

- i. Remove a portion of the sample and place into a clean porcelain dish.

- ii. Place 2mL of petroleum ether in to the porcelain dish to extract the sample.
- iii. Evaporate the solution to dryness using a hot plate.
- iv. Add 2mL of duquenois reagent to the porcelain dish and stir gently.
- v. Add 2mL of concentrated hydrochloric acid to the dish and stir gently. Let the solution stand for a few minutes and note if a color change develops.
- vi. Transfer the 1-2mL of the solution into a labeled culture tube and shake with 2mL of chloroform. Two discernable layers will form and note if a color change develops.
- vii. The results will be recorded on the Drug Analysis Form by documenting the actual color/s observed. The result is considered positive when both the Duquenois and Levine portions are observed and are specified in the notes. Negative observations will be recorded by stating no reaction or no color change

G. Interpretation

- i. For a positive Duquenois portion, a blue/purple color will develop.
- ii. For a positive Levine portion, the bottom layer will turn a pink/purple color.

H. Gas Chromatography Screen

- i. GC analysis will be performed on all suspected marijuana samples.
- ii. Weigh 25mg of sample and place into a labeled culture tube.
- iii. Add 5mL of methanol to the culture tube and vortex the solution. Allow the solution to stand for at least ½ hr.
- iv. Pipette 2mL of the methanolic extract into a labeled GC vial and cap tightly.
- v. Initiate auto sampler sequence using the ROUTINE method running a blank solvent between each unknown sample and reference standard/s.
- vi. Compare retention time of the each sample with the reference standard/s. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.
- vii. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

I. Criteria for Gas Chromatography

- i. Retention time of the sample must be within +/- 1.5% of the reference standard.
- ii. The concentration of the sample should be equivalent to the standard.

J. Gas Chromatography/Mass Spectrometry (as necessary)

- i. Confirmatory analysis will be performed if the results of any of the 4 prior tests are inconclusive/negative or if the net weight of the sample is \geq 50 lbs (pounds).
- ii. Confirmatory analysis can be performed using the GC vial from the previous section (H).
- iii. Initiate auto sampler sequence using the THC method running a blank solvent between each unknown sample and reference standard/s.
- iv. Compare retention time and ion spectra of the each sample with the reference standard/s (THC).
- v. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

K. Criteria for Gas Chromatography/Mass Spectrometry

- i. Retention time of the sample must be within +/- 1.5% of the reference standard.
- ii. Library spectra match must be $> 90\%$.
- iii. There must be a visual spectral match between the reference standard and the sample.
- iv. At least 5 of the major ions must be present for the sample.

L. Quantitation

- i. Quantitative analysis will be performed on suspected hashish samples that are $\geq 28\text{g}$ (grams).
- ii. Weigh and crush 20mg of sample and place into a labeled culture tube with 10mL of 0.45mg/mL ISTD (C). Allow the sample to soak overnight in the refrigerator.
- iii. Pipette 2mL of the sample into a labeled GC vial and cap tightly. Prepare 2 separate GC vials for analysis.
- iv. Prepare the standards as indicated in the reagent/standard preparation section. If the standards are already prepared, they must be at room temperature prior to use.
- v. Pipette out 0.8mL of each standard and place into individually labeled residue vial.
- vi. Initiate auto sampler sequence using the THCQUANT method running methanol blank solvent between each reference standard/s.
- vii. Check the concentration of each standard to determine if it meets the criteria of the laboratory.
- viii. If standards are acceptable, continue with the analysis. If any of the standards are out of range (spec), notify the lab supervisor (make up new standards).
- ix. Initiate auto sampler sequence using the THCQUANT method running methanol blank solvent between each reference standard/s.
- x. Sequence order should be similar: Blank, 1.0 standard, blank, 0.4 standard, blank, sample-1, blank, sample-2, blank, 0.2 standard, blank, 1.0 standard, and blank.
- xi. For sample: take the average of both results.
- xii. Document the results on the Quantitation Analysis Form, Drug Analysis Form and Control Card.

M. Criteria for Quantitation

- i. The concentration of each standard must be within
- ii. Results: If $\% \text{THC} > 2.5\%$ report as Class C. If $\% \text{THC} \leq 2.5\%$ report as Class D.

11. Purity Calculations

$$\% \text{ Drug} = \frac{(\text{STD}) \times R2 \times V \times 100}{R1 \times W}$$

(STD) = concentration of calibration standard in mg/ml

$$R2 = \frac{\text{peak area (height) of sample}}{\text{peak area (height) of internal standard}}$$

$$R1 = \frac{\text{peak area (height) of standard}}{\text{peak area (height) of internal standard}}$$

V = volume of internal standard solution used in ml

W = sample weight in mg

12. Documentation

A. All results will be documented on the Drug Analysis Form.

{ DATE \@ "M/d/yyyy" }

- B. All raw data will be generated and filed according to the laboratory policy.
- C. A certificate of analysis will be generated for each lab number which will document the results.

13. Attachments

GC Method

GC/MS Method